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Confocal microscopy from the bench to the bedside

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Confocal microscopy is a powerful tool for performing high-resolution imaging in optically thick biologic tissue using the process of optical sectioning.¹ The basic principle is shown in Figure 1, where light emerging from a fiber is focused by an objective lens into the tissue. Scattered light originating from the in-focus plane converges back into the fiber, whereas light from the out-of-focus planes is inefficiently collected. Confocal microscopy is sensitive to fluorescence, unlike optical coherence tomography, another method being developed for performing optical sectioning and, thus, can provide greater image contrast and observe mucosal function. Conventional confocal microscopes are large laboratory instruments that use complex, high numerical aperture objectives to achieve subcellular resolution. Previously, the primary challenge that has limited the in vivo use of confocal microscopy was the ability to reduce the optics and the scanning mechanism to an endoscope-compatible size. In this issue of *Gastrointestinal Endoscopy*, Polgase et al² describe the use of a novel, confocal imaging prototype that uses 488-nm illumination to produce fluorescence images from normal esophagus, antrum, duodenum, ileum, colon, and rectum in vivo. These images reveal sharp cellular and subcellular details that provide a striking comparison with corresponding histology.

Confocal microendoscopy provides powerful imaging features that complement instead of replace the use of conventional white-light endoscopy. As shown by the comparison in Table 1, standard endoscopy images the mucosal surface with relatively low resolution over a large field of view that can visualize the entire surface area of target organs in a few minutes. Adaptations are being developed to enhance the ability of endoscopy to detect disease and to include autofluorescence endoscopy, narrow-band imaging, and chromoendoscopy. On the other hand, confocal microendoscopy visualizes below the tissue surface with subcellular resolution over a much smaller region of tissue and can be considered as point detection. As confocal instrumentation technology advances, a new frontier of GI imaging will continue to emerge. As shown in this paper,² the investigators demonstrated the use of confocal microscopy to perform optical biopsy, a non-destructive in situ method of performing instantaneous mucosal histopathology without the risk of bleeding. Intravenous fluorescein and topical acriflavine are used to enhance image contrast in a manner analogous to the use of H&E in histology. The primary clinical end points of an optical biopsy are to target premalignant mucosa for excision, to grade and stage cancer progression, and to reduce the risk of physical biopsy.

Confocal microendoscopy is expected to go beyond in vivo histopathology to dynamic physiologic and molecular biologic measurements and create new frontiers in GI endoscopy.

Whereas, the prototype described in this paper represents a significant achievement, further technologic advancements are needed to reach the full potential of confocal microendoscopy. First, optical sectioning at greater depths is necessary. While an axial scan range of 200 μ m is reported, the images shown appear to have been collected near the mucosal surface. More tissue penetration is needed to image the full mucosa depth, which is closer to 300 to 400 μ m for normal mucosa and may extend beyond 1 mm with disease. These levels of depth can be achieved with the use of infrared light and would be needed to identify submucosal tumor invasion and micrometastases. Next, the development of contrast agents specific for disease is

needed. While the use of fluorescein and acriflavine in this study is effective for enhancing image contrast in normal mucosa, their use with disease may be limited. Fluorescein distributes through the microvasculature and, thus, lacks specificity and may extravasate in the presence of inflammation. Moreover, acriflavine is a known carcinogen; thus, it may not be suitable for routine use. Greater disease specificity may be possible with the administration of molecular probes that bind to biomarkers of disease, such as high-affinity peptide and small molecule reagents. Furthermore, increased frame rates are desirable. Whereas, image collection at 1 second/frame is sufficient for observing optical biopsy, faster acquisition would allow for dynamic observation of mucosal behavior, such as cell-cell and cell-protein interactions. A factor of 10 or more improvement is possible by scanning the laser beam with a microelectromechanical systems mirror to overcome limitations in scanning speed imposed by the inertia of the fiber.³

The potential future clinical impact of confocal microendoscopy extends far beyond that of in vivo histopathology alone, which represents merely a single snapshot in time of tissue morphology. The real-time imaging capabilities of confocal microscopy allow for dynamic physiologic measurements to be made, which until now was not possible. Moreover, the sensitivity of confocal microscopy to fluorescence provides an opportunity to study the molecular biology of the mucosa by using specific probes. This methodology can be combined with multispectral imaging to form a very powerful tool. These features open the door to a new frontier of GI imaging for investigating biologic behavior that occurs in its native environment. Previously unseen mucosal processes, such as the response to environmental stimulants (e.g., acid, bile, and gluten), lymphocyte trafficking, tumor growth and migration, and wound healing, may soon be studied. Furthermore, clinical monitoring of therapeutic interventions, such as mucosal uptake and transport of drugs, measurement of tissue pharmacology, and image guidance of photodynamic ablation, may soon be performed. In this paper, Polgase et al² have demonstrated the potential for performing biologic investigations at the bedside, which previously have been conducted exclusively at the bench, thus, providing a glimpse of the wealth of biologic information that may soon be available for medical decision making.

References

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Figure 1.

With confocal imaging, light that emerges from the fiber is focused by the objective lens into the tissue and scattered light originating from the in-focus plane is refocused into the fiber; whereas, light emanating from the out-of-focus plane is rejected.

TABLE 1

Comparison of imaging parameters between conventional white-light endoscopy and confocal microendoscopy

Imaging parameters	White light endoscopy	Confocal microscopy
Transverse resolution, mm	~100	~1
Axial resolution, mm	—	~7
Field of view, mm	~100	~0.5
Frame rate (per sec)	30	~1
Tissue penetration depth, mm	~0	0–250
Image contrast	Endogenous	Exogenous